



This form should be used for all taxonomic proposals. Please complete all those modules that are applicable (and then delete the unwanted sections). For guidance, see the notes written in blue and the separate document "Help with completing a taxonomic proposal"

Please try to keep related proposals within a single document; you can copy the modules to create more than one genus within a new family, for example.

MODULE 1: **TITLE, AUTHORS, etc**

Code assigned:	2016.055aB	(to be completed by ICTV officers)			
Short title: To add three (3) new species to the genus, <i>Wphvirus</i> , in the family <i>Myoviridae</i> . (e.g. 6 new species in the genus <i>Zetavirus</i>)					
Modules attached (modules 1 and 10 are required)	1 <input checked="" type="checkbox"/>	2 <input checked="" type="checkbox"/>	3 <input type="checkbox"/>	4 <input type="checkbox"/>	5 <input type="checkbox"/>
	6 <input type="checkbox"/>	7 <input type="checkbox"/>	8 <input type="checkbox"/>	9 <input type="checkbox"/>	10 <input checked="" type="checkbox"/>

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List the ICTV study group(s) that have seen this proposal:

A list of study groups and contacts is provided at <http://www.ictvonline.org/subcommittees.asp> . If in doubt, contact the appropriate subcommittee chair (fungal, invertebrate, plant, prokaryote or vertebrate viruses)

ICTV Bacterial and Archaeal Viruses Subcommittee

ICTV Study Group comments (if any) and response of the proposer:

Date first submitted to ICTV: June 2016
Date of this revision (if different to above):

ICTV-EC comments and response of the proposer:

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MODULE 2: **NEW SPECIES**

creating and naming one or more new species.

If more than one, they should be a group of related species belonging to the same genus. All new species must be placed in a higher taxon. This is usually a genus although it is also permissible for species to be “unassigned” within a subfamily or family. Wherever possible, provide sequence accession number(s) for **one** isolate of each new species proposed.

Code	2016.055aB	(assigned by ICTV officers)
To create 3 new species within:		
Genus:	<i>Wphvirus</i>	Fill in all that apply. • If the higher taxon has yet to be created (in a later module, below) write “ (new) ” after its proposed name. • If no genus is specified, enter “ unassigned ” in the genus box.
Subfamily:		
Family:	<i>Myoviridae</i>	
Order:	<i>Caudovirales</i>	
Name of new species:	Representative isolate: (only 1 per species please)	GenBank sequence accession number(s)
<i>Bacillus virus BPS13</i>	Bacillus phage BPS13	JN654439.1
<i>Bacillus virus Megatron</i>	Bacillus phage Megatron	KJ489401.1
<i>Bacillus virus Hakuna</i>	Bacillus phage Hakuna	KJ489399.1

<p>Reasons to justify the creation and assignment of the new species:</p> <ul style="list-style-type: none"> Explain how the proposed species differ(s) from all existing species. <ul style="list-style-type: none"> If species demarcation criteria (see module 3) have previously been defined for the genus, explain how the new species meet these criteria. If criteria for demarcating species need to be defined (because there will now be more than one species in the genus), please state the proposed criteria. Further material in support of this proposal may be presented in the Appendix, Module 9 <p>We have chosen 95% DNA sequence identity as the criterion for demarcation of species in this new genus. The members of each of the proposed species differ from those of other species by more than 5% at the DNA level as confirmed with the BLASTN algorithm.</p>
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MODULE 10: **APPENDIX**: supporting material

additional material in support of this proposal

References:

<ol style="list-style-type: none"> Darling AE, Mau B, Perna NT. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One. 2010; 5(6):e11147. Turner D, Reynolds D, Seto D, Mahadevan P. CoreGenes3.5: a webserver for the determination of core genes from sets of viral and small bacterial genomes. BMC Res Notes. 2013; 6:140. doi: 10.1186/1756-0500-6-140. Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, Dufayard JF, Guindon
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additional material in support of this proposal

References:

- S, Lefort V, Lescot M, Claverie JM, Gascuel O. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. *Nucleic Acids Res.* 2008; 36(Web Server issue):W465-9.
4. Park J, Yun J, Lim JA, Kang DH, Ryu S. Characterization of an endolysin, LysBPS13, from a *Bacillus cereus* bacteriophage. *FEMS Microbiol Lett.* 2012;332(1):76-83. [BPS13]
5. Erill I, Caruso SM. Genome Sequences of Two *Bacillus cereus* Group Bacteriophages, Eyuki and AvesoBmore. *Genome Announc.* 2015;3(5). pii: e01199-15. [Eyuki]
6. Asare PT, Jeong TY, Ryu S, Klumpp J, Loessner MJ, Merrill BD, Kim KP. Putative type 1 thymidylate synthase and dihydrofolate reductase as signature genes of a novel Bastille-like group of phages in the subfamily *Spounavirinae*. *BMC Genomics.* 2015;16:582.

Annex:

Include as much information as necessary to support the proposal, including diagrams comparing the old and new taxonomic orders. The use of Figures and Tables is strongly recommended but direct pasting of content from publications will require permission from the copyright holder together with appropriate acknowledgement as this proposal will be placed on a public web site. For phylogenetic analysis, try to provide a tree where branch length is related to genetic distance.

Comment: In “Create the *Wphvirus* genus including 1 species within the family *Myoviridae* (2015.041a-dB)” we stated “Phylogenetic analysis (6) of the major capsid (Fig. 3) and large subunit terminase (Fig. 4) reveal that phage W.Ph. is peripherally related to phages BPS13, Hakuna and Megatron; while analysis of the DNA polymerase (Fig. 5) suggests these phages form a clade.” A re-examination of the data in light of recent finding by Asare *et al.* [6] and unpublished findings by J. Rodney Brister and Igor Tolstoy from NCBI, coupled with the presence of high numbers of homologous proteins (Table 1), calls for the inclusion of these phages in the *Wphvirus* genus.

Table 1. Properties of the two phages belonging to the genus *Wphvirus*.

Bacillus phage	RefSeq No	GenBank Accession No.	Genome length (kb)	Genome (mol% G+C)	No. CDS	DNA (% sequence identity)*	% Homologous proteins **
W.Ph.	NC_016563.1	HM144387.1	156.90	36.4	274	100	100
BPS13	NC_018857.1	JN654439.1	158.31	38.8	268	70	86.1
Megatron	NC_024211.1	KJ489401.1	158.75	38.8	290	66	91.6
Hakuna	NC_024213.1	KJ489399.1	158.10	38.7	294	66	91.2

* Determined using BLASTN; ** Determined using CoreGenes [2]; Bacillus phage Eyuki (KT207918.1) should be considered a strain of Bacillus phage Megatron, while Bacillus phage BPS10C (KC430106.1) is a strain of Bacillus phage BPS13 within this genus.

Fig. 2. progressiveMauve alignment [1] of the annotated genomes of members of the *Wphvirus* genus – from top to bottom: Bacillus phages W.Ph., BSP13, Hakuna, Megatron. Colored blocks indicate the regions of 1 to 1 best alignment with rearrangement breakpoints in a different random color. The degree of sequence similarity between regions is given by a similarity plot within the colored blocks with the height of the plot proportional to the average nucleotide identity (Aaron Darling, personal communication). N.B. The cursor indicates that none of these phage genomes is collinear with that of W.Ph.

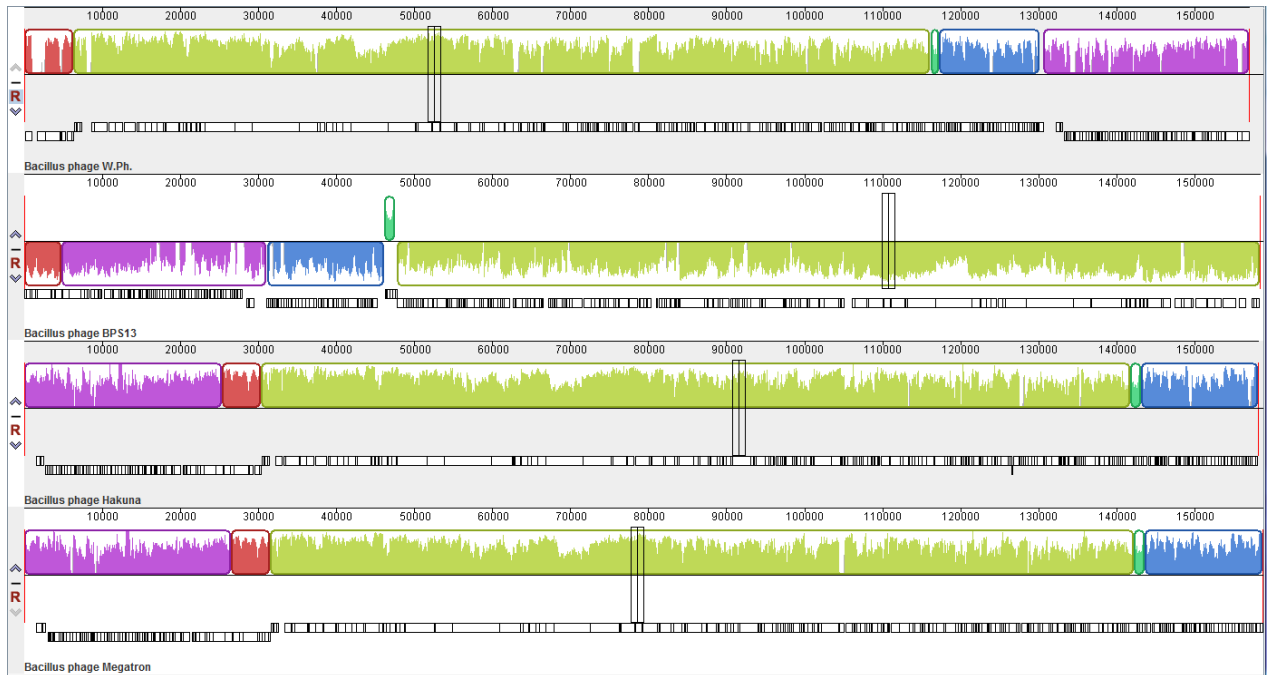


Fig. 3. Phylogenetic analysis of **A.** major capsid protein, **B.** large subunit terminase proteins and **C.** metallophosphatases of Bacillus phage W.Ph.-like viruses and homologous proteins from a variety of other phages constructed using “one click” at phylogeny.fr [3]. "The "One Click mode" targets users that do not wish to deal with program and parameter selection. By default, the pipeline is already set up to run and connect programs recognized for their accuracy and speed (MUSCLE for multiple alignment and PhyML for phylogeny) to reconstruct a robust phylogenetic tree from a set of sequences." It also includes the use of Gblocks to eliminate poorly aligned positions and divergent regions. "The usual bootstrapping procedure is replaced by a new confidence index that is much faster to compute. See: Anisimova M., Gascuel O. Approximate likelihood ratio test for branches: A fast, accurate and powerful alternative (Syst Biol. 2006;55(4):539-52.) for details." New genus is boxed in **green**.

A. Major capsid protein

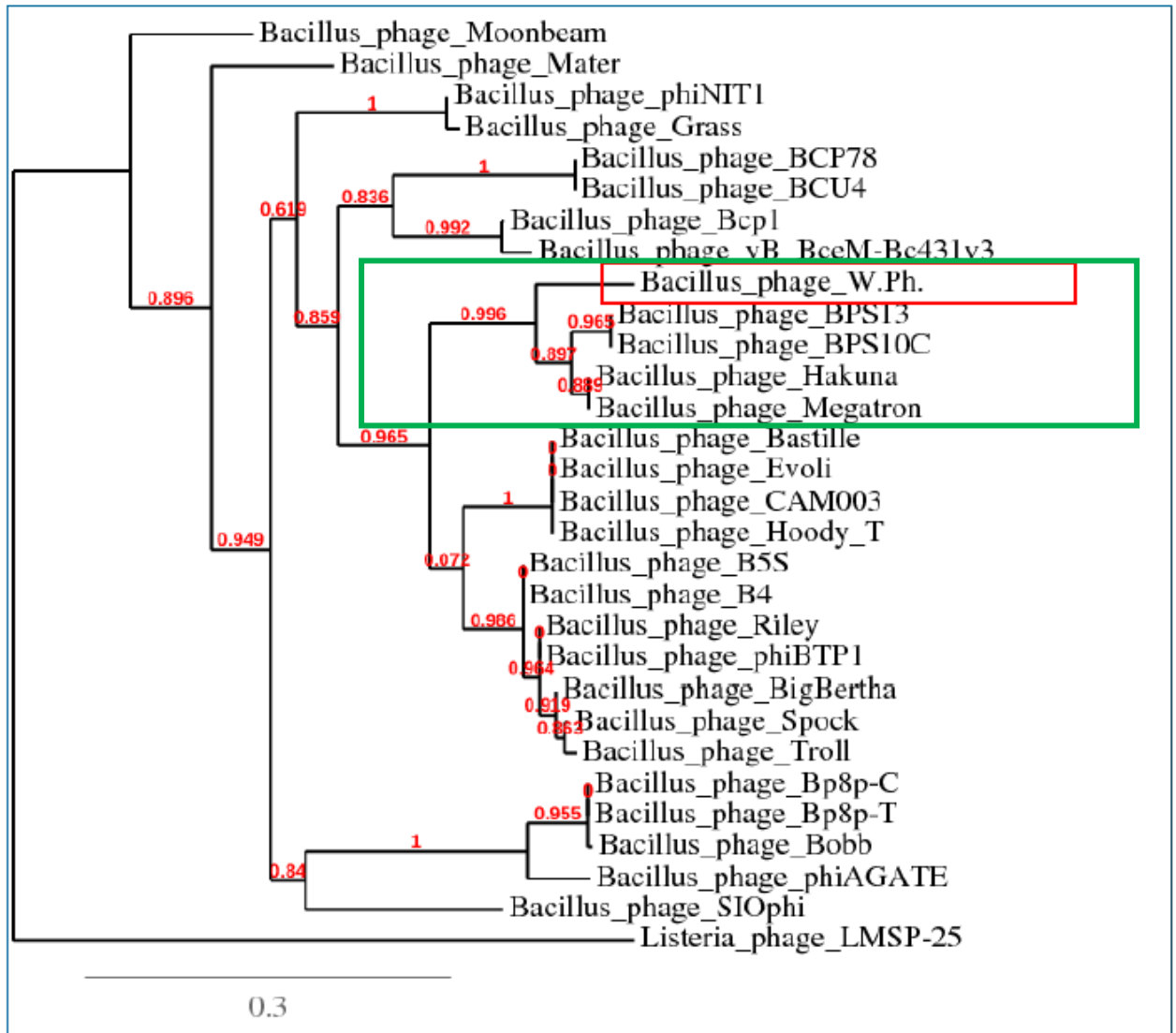


Figure 1: Phylogenetic tree (the branch length is proportional to the number of substitutions per site).

B. Terminase, large subunit

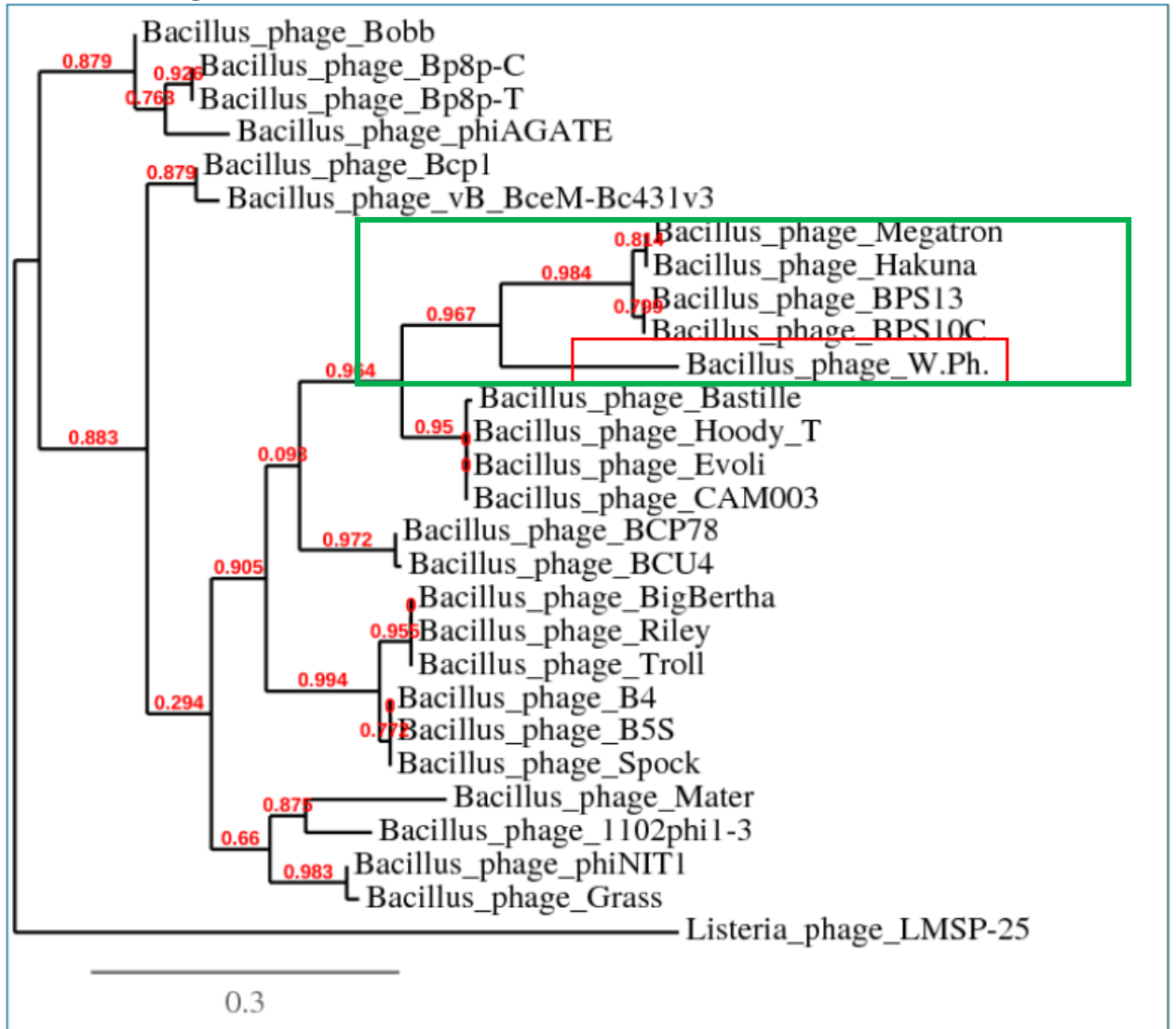


Figure 1: Phylogenetic tree (the branch length is proportional to the number of substitutions per site).

C. Metallophosphatases

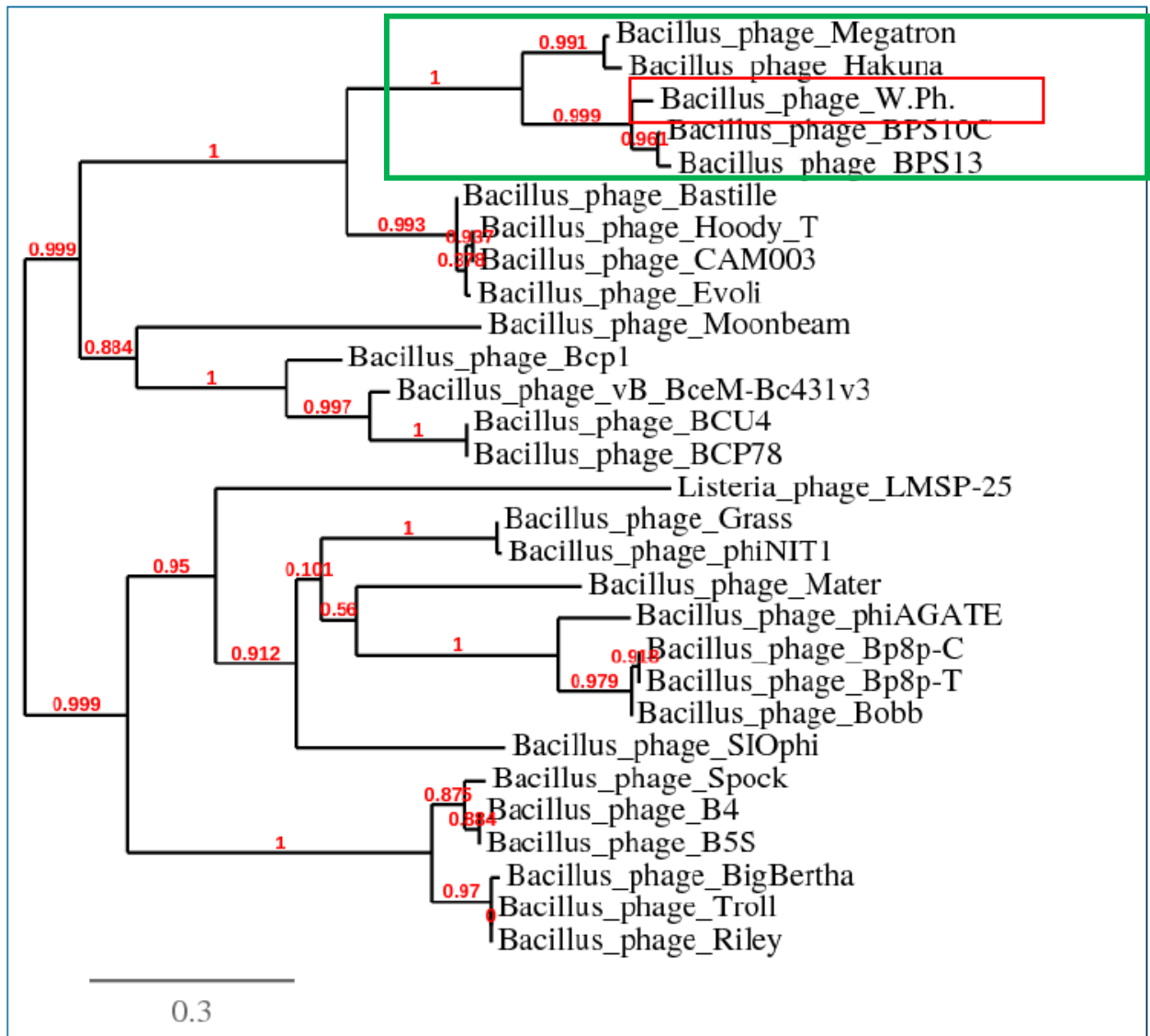


Figure 1: Phylogenetic tree (the branch length is proportional to the number of substitutions per site).